Received: 10 October 2022 | Revised: 16 December 2022 | Accepted: 21 December 2022

DOI: 10.1097/HEP.0000000000000300

ORIGINAL ARTICLE

Plasma proteomic signature of fatty liver disease: The Rotterdam Study

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Abstract

Background and Aims: Fatty liver disease (FLD) is caused by excess fat in the liver, and its global prevalence exceeds 33%. The role of protein expression on the pathogenesis of FLD and accompanied fibrosis and its potential as a disease biomarker is currently not clear. Hence, we aimed to identify plasma proteomics associated with FLD and fibrosis using population-based data.

Approach and Results: Blood samples were collected from 2578 participants from the population-based Rotterdam Study cohort. The proximity extension assay reliably measured plasma levels of 171 cardiometabolic and inflammatory-related proteins (Olink Proteomics). FLD was assessed by ultrasound, and fibrosis by transient elastography. Logistic regression models quantified the association of plasma proteomics with FLD and fibrosis. In addition, we aimed to validate our results in liver organoids. The cross-sectional analysis identified 27 proteins significantly associated with FLD surpassing the Bonferroni-corrected $p < 2.92 \times 10^{-4}$. The strongest association was observed for FGF-21 ($\beta = 0.45$, $p = 1.07 \times 10^{-18}$) and carboxylesterase 1 (CES1) protein ($\beta = 0.66$, $p = 4.91 \times 10^{-40}$). Importantly, 15 of the 27 proteins significantly associated with FLD were also associated with liver fibrosis. Finally, consistent with plasma proteomic profiling, we found the expression levels of IL-18 receptor 1 (IL-18R1) and CES1 to be upregulated in an FLD model of 3-dimensional culture human liver organoids.

Abbreviations: CCL19, chemokine ligand 19; CDCP1, CUB domain-containing protein 1; CES1, carboxylesterase 1; CHL1, close homolog of L1; CNDP1, beta-Ala-His dipeptidase; CX3CL1, chemokine ligand 1; F7, coagulation factor VII; FCN2, ficolin-2; FIB-4, fibrosis-4; FLD, fatty liver disease; FLI, fatty liver index; HVEM, herpes simplex virus glycoprotein D for herpes virus entry mediator; ICAM1, intercellular adhesion molecules 1; IL-18R1, IL-18 receptor 1; KIT, proto-oncogene c-KIT; LILRB1, leukocyte immunoglobulin-like receptor subfamily B member 1; MAFLD, metabolic dysfunction-associated fatty liver disease; MEGF9, multiple EGF-like-domains 9; Met, tyrosine-protein kinase; PANTHER, protein annotation through evolutionary relationship; PLTP, phospholipid transfer protein; PROC, protein C; PTPRS, receptor-type tyrosine-protein phosphatase precursor; SCF, stem cell factor; SERPINA5, serine protease inhibitor 5; SERPINA7, serine protease inhibitor 7; TGFBI, transforming growth factor, beta-induced, 68kDa; TGFBR3, transforming growth factor beta receptor III; THBS4, thrombospondin-4; TNFSF14, tumor necrosis factor superfamily member 14; TWEAK, TNF-related weak inducer of apoptosis.

Ibrahim Ayada and Laurens A. van Kleef contributed equally to this work.

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Conclusions: Among the general population, several inflammatory and cardiometabolic plasma proteins were associated with FLD and fibrosis. Particularly, plasma levels of FGF-21, IL-18R1, and CES1 were largely dependent on the presence of FLD and fibrosis and may therefore be important in their pathogenesis.

INTRODUCTION

Fatty liver disease (FLD) has become increasingly prevalent in the past decades, with over 33% of the world's population currently affected.[1] This worrisome rise in prevalence has followed an increase in obesity and other metabolic disorders.[2] FLD is a spectrum disease ranging from simple steatosis to more severe consequences like steatohepatitis, cirrhosis, and even HCC.[3–5] Despite the high prevalence and enormous disease burden, the understanding of protein expression in FLD and fibrosis is limited, as well as their potential as biomarkers to facilitate early detection.[6,7]

Proteomics is a powerful tool for examining differences in protein expression. Promising results have been obtained previously by using sophisticated techniques to quantify protein expression. Recently, a range of proteins has been identified that were crucial for the presence and severity of the alcohol-associated liver disease.[8] This indicates that proteomic signatures may act as potential biomarkers to predict FLD at an early stage. Indeed several small studies already showed its value using the SomaScan assay to define protein expression in patient cohorts with advanced FLD.[9,10]

Given the potential of proteomics, we performed plasma profiling of inflammatory and cardiometabolic-related proteins among a large cohort of community-dwelling individuals and thereby aimed to increase the understanding of protein expression in FLD and liver fibrosis, as well as assess their potential as disease biomarkers.

METHODS

Participants

This study was conducted within the prospective Rotterdam Study cohort targeting community-dwelling individuals aged 45 years or above living in Ommoord, a suburb of Rotterdam. The design, aims, and recent achievements of the Rotterdam Study have extensively been described elsewhere.[11] For the current study, we included participants from the third Rotterdam Study extension, collected between February 2006 and December 2008, with available data on the plasma levels of inflammatory ($n = 3456$) and cardiometabolic-related proteins ($n = 3502$). Exclusion criteria were missing data on FLD or covariates included in the final models (Figure 1). The final sample size for the inflammatory panel was 2578, and for the cardiometabolic panel, 2595.

Assessment of FLD and liver stiffness

FLD was defined as hyperechoic liver parenchyma compared with the kidney cortex, which was assessed by abdominal ultrasonography between 2009 and 2014, 5.5 (P25–P75: 5.4–5.7) years after plasma sample collection.[12] A single experienced sonographer performed all the examinations on a Hitachi HI VISION 900. In addition to FLD, we defined NAFLD and metabolic dysfunction-associated fatty liver disease (MAFLD) for sensitivity analysis. NAFLD was defined as the presence of FLD in the absence of the following secondary causes of FLD: (1) excessive alcohol consumption (>30 g/d for men and >20 g/d for women), (2) presence of viral hepatitis, and (3) use of steatogenic drugs. MAFLD was defined as FLD combined with metabolic dysfunction, comprising overweight (BMI ≥ 25 kg/m2), type 2 diabetes mellitus, or 2 minor metabolic dysfunction criteria, such as hypertension, high waist circumference, or dyslipidemia.[13] Moreover, liver stiffness measurement was performed using transient elastography (FibroScan; Echosens, Paris, France). At least 10 measurements were obtained with the M or XL probe. In the case of an interquartile range of $>30\%$, measurements exceeding 7.1 kPa were considered unreliable and discarded.[14] Fibrosis was defined as a reliable liver stiffness measurement of ≥ 8.0 kPa.[15] Plasma proteomic profiling was performed before the assessment of FLD and fibrosis.

Assessment of plasma proteomic profiling

Plasma protein levels were measured using the proximity extension immunoassay technology (Olink Proteomics, Uppsala, Sweden) on the ProSeek Multiplex Inflammation (version 3021) and Cardiometabolic

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(version 3602) 96-plex panels. The Olink proximity extension immunoassay technology uses a dual recognition DNA-coupled immunoassay that rapidly allows protein identification and relative quantification with high sensitivity and specificity. The method of proteomics level measurement has been described in detail.[16]

Olink-generated proteomics data were background corrected, log2 transformed, and normalized to a Normalized Protein Expression (NPX) scale. NPX values represent relative quantification, meaning that protein values can be compared for the same protein across samples.

Sample-based Quality Control (QC) of the proteomics data was done as published [17] based on Olink-reported QC warnings, that is, samples that deviate $> \pm 0.3$ NPX from the plate median. In this study, there were a total of 76 QC warnings. For assay-based QC, we excluded proteins with a high proportion of samples ($> 90\%$) with NPX values below the limit of detection. For the inflammation panel, 9 proteins were removed, including IL-1α, IL-2, IL-24, IL-20, IL-33, leukemia inhibitory factor, thymic stromal lymphopoietin, neurturin, NRTN, and beta-nerve growth factor (NRTN). Although 4 proteins were removed from the cardiometabolic panel, including lysosomal Pro-X carboxypeptidase, latent TGF beta-binding protein 2 (LTBP2), lithostathine-1-alpha (REG1A), and superoxide dismutase (SOD1). NPX values below the limit of detection were treated as missing values. After QC, we retained 83 proteins from the inflammation panel and 88 proteins from the cardiometabolic panel.

Assessment of covariates

Information on age, sex, smoking status (3 categories current/former/never), and alcohol consumption (measured in grams of ethanol per day) were obtained from questionnaires.[11] Body mass index was calculated based on the weight in kilograms divided by the height in meters squared. Prevalent diabetes mellitus type 2 was identified according to the World Health Organization criteria: fasting glucose levels of ≥ 7.0 mmol/L, nonfasting glucose levels ≥ 11.1 mmol/L, or the use of glucose-lowering medication.[18] Fasting blood samples of participants were obtained during each visit to the research center. Full blood count measurements were performed directly after the blood sample was drawn using the COULTER Ac·T diff2 Hematology Analyzer (Beckman Coulter, Brea, CA). Laboratory measurements included absolute granulocyte, platelet, lymphocyte, and monocyte counts in 10^9 per liter. All covariates included for statistical analysis were obtained at baseline examination.

Validation in human liver organoids

A database was designed and recently published by our NAFLD/NASH group containing results on genome-wide transcriptomic profiling on human liver organoids mimicking FLD compared with controls. Detailed information on the procedures is available elsewhere.[19]

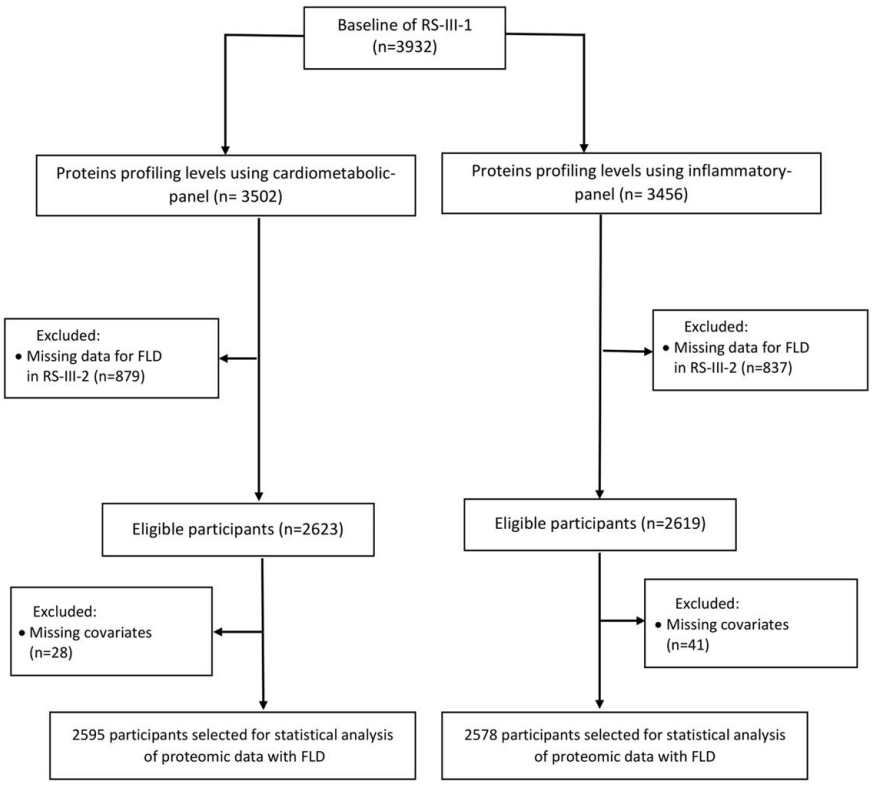
Baseline of RS-III-1 (n=3932)
Proteins profiling levels using cardiometabolic-panel (n= 3502)
Proteins profiling levels using inflammatory-panel (n= 3456)
Excluded:
• Missing data for FLD in RS-III-2 (n=879)
Excluded:
• Missing data for FLD in RS-III-2 (n=837)
Eligible participants (n=2623)
Eligible participants (n=2619)
Excluded:
• Missing covariates (n=28)
Excluded:
• Missing covariates (n=41)
2595 participants selected for statistical analysis of proteomic data with FLD
2578 participants selected for statistical analysis of proteomic data with FLD

FIGURE 1 Flow chart of the study participants. Abbreviations: FLD, fatty liver disease; RS-III, the third cohort of Rotterdam Study.

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TABLE 1 Characteristics of the Rotterdam Study participants

Characteristics	FLD-US		Liver fibrosis-TE	
	Case	Control	Case	Control
N	907	1671	105	2157
Age	56.72 ± 5.68	56.14 ± 5.94	57.85 ± 7.03	56.05 ± 5.63
Sex, Female, n (%)	465 (51.3)	994 (59.5)	31 (29.5)	1235 (57.3)
Body mass index (kg/m^2)	29.68 ± 4.40	26.51 ± 3.96	29.62 ± 4.77	27.21 ± 4.03
Alcohol consumption (g/d)	9.89 ± 10.65	8.08 ± 8.53	11.52 ± 10.24	8.90 ± 9.23
Type 2 diabetes, n (%)	136 (15.0)	92 (5.5)	27 (25.7)	147 (6.8)
Smoking status, n (%)				
Never	262 (28.9)	589 (35.2)	25 (23.8)	727 (33.7)
Current	183 (20.2)	357 (21.4)	27 (25.7)	452 (21.0)
Former	462 (50.9)	725 (43.4)	53 (50.5)	978 (45.3)
Blood cells counts, 10^9/L				
Red blood cells	5.09 ± 0.35	4.80 ± 0.37	5.07 ± 0.38	4.87 ± 0.37
Granulocyte	4.17 ± 1.40	4.01 ± 1.46	4.19 ± 1.33	4.05 ± 1.46
Lymphocyte	2.45 ± 0.66	2.46 ± 0.72	2.50 ± 0.70	2.44 ± 0.69
Monocyte	0.43 ± 0.22	0.43 ± 0.23	0.43 ± 0.16	0.43 ± 0.23
Platelets	0.46 ± 0.03	0.44 ± 0.03	0.46 ± 0.03	0.44 ± 0.03

Note: The characteristics of study participants in both inflammatory and cardiometabolic proteomics panels; Variables are represented as mean (± SD), or number (%).
Abbreviations: FLD, fatty liver disease; N, sample size; TE, transient elastography; US, ultrasound.

Within this database, we aimed to validate our results with the genome-wide transcriptomic profiling from this experimental model.

Statistical analysis

We used multivariable linear and logistic regression models to quantify the association between plasma levels of proteins as exposure and (1) FLD and (2) liver stiffness (continuous and categorical) as the outcome. The associations were adjusted for age and sex (model 1) and, in addition, for blood cell counts (RBCs, granulocyte, lymphocyte, monocyte, and platelet), BMI, alcohol, diabetes, and smoking (model 2). In sensitivity analysis, we replaced FLD with NAFLD and MAFLD. Additional sensitivity analysis was performed using a fatty liver index (FLI) based on the definition of FLD. The FLI comprised BMI, waist circumference, gamma-glutamyltransferase, and triglycerides,[20] which were collected at the same time as plasma sample collection.

Further analysis focused on the potential of protein expression as a biomarker for FLD and fibrosis. The AUC was calculated, including the CIs for the set of significantly associated proteins in the prediction of FLD and fibrosis, based on a logistic regression model including the significantly associated proteins previously identified. The diagnostic accuracy of proteins was then compared with the common noninvasive markers for the prediction of FLD and fibrosis. These include FLI as a marker for FLD and the fibrosis-4 index (FIB-4), comprised of age, aspartate transaminase, alanine transaminase, and platelets, as a marker for fibrosis. Finally, proteomics was combined with FLI or FIB-4 to assess the added value of proteins for already existing and commonly used scores.

All analyses were performed using SPSS statistical software (SPSS, version 25; IBM Corp) and R software version 3.5.2 (The R Foundation for Statistical Computing). To account for the multiple testing, the Bonferroni adjusted *p*-value for our primary aim (protein expression with FLD) was 2.92×10^{-4} based on the number of tested proteins (n = 171) and set as the threshold for statistical significance. The PANTHER (v.17.0) (protein annotation through evolutionary relationship) classification system (http://www.pantherdb.org/) was used to check the Reactome-pathways of the identified proteins in both inflammatory and cardiometabolic panels.

ETHICS

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC University Medical Center (registration number MEC 02.1015) and the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study Personal Registration Data collection is filed with the Erasmus MC Data Protection Officer under registration number

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EMC1712001. The Rotterdam Study has been entered into the Netherlands National Trial Register (http://www.trialregister.nl/) and into the World Health Organization International Clinical Trials Registry Platform (http://www.who.int/ictrp/network/primary/en/) under shared catalog number NTR6831. All participants provided written, informed consent to participate in the study and to have their information obtained from treating physicians. All authors had access to the study data and reviewed and approved the final manuscript.

RESULTS

Participant characteristics

The baseline characteristics of study participants are illustrated in Table 1 and S1 (http://links.lww.com/HEP/C647). A total of 2578 participants with available data on plasma protein levels and abdominal ultrasound were included. In general, the mean age was 56.3 ± 5.9 years, 56.6% were female and metabolic comorbidity was highly common, illustrated by the mean BMI of 27.6 kg/m^2 ± 4.4 and diabetes prevalence of 8.8%. The overall prevalence of FLD was 35.2%, and 4.6% had fibrosis.

Plasma proteins associated with FLD

We found 8 out of 83 inflammatory-related proteins to be significantly associated with FLD in fully adjusted models, taking into account multiple testing corrections ($p < 2.92 \times 10^{-4}$). Among them, 5 proteins [FGF-21, HGF, CUB domain-containing protein 1 (CDCP1), TNF superfamily member 14 (TNFSF14), and IL-18R1] were positively associated, whereas 3 proteins [chemokine ligand 1 (CX3CL1), stem cell factor (SCF), and TNF-related weak inducer of apoptosis (TWEAK)] were negatively associated with FLD (Table 2A and Figure 2A). Regarding the cardiometabolic panel, we identified 19 out of 88 proteins to be significantly associated with FLD. Of these, 12 proteins [carboxylesterase 1 (CES1), coagulation factor VII (F7), serine protease inhibitor 5 (SERPINA5), thrombospondin-4 (THBS4), protein C (PROC), ficolin-2 (FCN2), intercellular adhesion molecules 1 (ICAM1), beta-Ala-His dipeptidase (CNDP1), Multiple EGF-like-domains 9 (MEGF9), leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1), transforming growth factor, beta-induced, 68kDa (TGFBI), and serine protease inhibitor 7 (SERPINA7)] were positively associated, and 7 proteins [tyrosine-protein kinase (MET), proto-oncogene c-KIT (KIT), phospholipid transfer protein (PLTP), receptor-type tyrosine-protein phosphatase precursor (PTPRS), a close homolog of L1 (CHL1), transforming growth factor beta receptor III (TGFBR3), and REG1A] were negatively associated with FLD (Table 2B and Figure 2B). Importantly, 25/27 proteins significantly associated with ultrasound-defined FLD could be validated using FLD based on FLI, which was calculated at the same time point as the proteomic data (Table S2 http://links.lww.com/HEP/C647).

Plasma proteins associated with fibrosis

Our analysis showed 7 out of the 8 inflammatory proteins significantly associated with FLD to be also associated with fibrosis (on a categorical scale ≥ 8.0 kPa) and liver stiffness (on a continuous scale). For the cardiometabolic panel, 8 out of the 19 proteins significantly associated with FLD were associated with fibrosis, and 10 out of the 19 proteins were associated with liver stiffness on a continuous scale in fully adjusted models (Table 2 and Table S3 http://links.lww.com/HEP/C647).

Association of plasma proteins with MAFLD and NAFLD

In our sensitivity analysis, consistent results were obtained when MAFLD replaced FLD. In addition, despite a drop in the sample size, after replacing FLD with NAFLD, the result remained significant ($p < 2.92 \times 10^{-4}$) in all 8 inflammatory proteins while the result for 4 of the 19 cardiometabolic-related proteins lost statistical significance (Table S4 and S5 http://links.lww.com/HEP/C647). It should be noted that despite the loss of statistical significance, similar direction of effects was observed in these 4 proteins as well.

Pathway analysis for proteins associated with FLD

We then performed the Reactome-pathways analysis, which aims to extract information on proteins through data mining and literature enrichment analysis. We arranged the pathways by fold-enrichment values and selected for further analysis with fold changes of > 9.5. We found TWEAK and TNFS14 proteins to be involved in the noncanonical NF-κB pathway (Table S6A, http://links.lww.com/HEP/C647). In addition, F7, SERPINA5, and PROC proteins were involved in the formation of fibrin clot (clotting cascade) (Table S6B http://links.lww.com/HEP/C647).

Validation of associations in liver organoids

In addition, we aimed to validate the results regarding protein expression and FLD using our *in vitro* data on

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TABLE 2 Plasma proteins significantly associated with FLD and liver fibrosis

	Fatty liver disease			Liver fibrosis		
Proteins ID	**Beta**	**SE**	***p***	**Beta**	**SE**	***p***
A—Inflammatory-related proteins						
FGF-21	0.45	0.051	1.07×10^{-18}	0.31	0.112	6.09×10^{-03}
HGF	0.34	0.055	1.27×10^{-09}	0.50	0.113	8.58×10^{-06}
CX3CL1	−0.23	0.048	1.63×10^{-06}	−0.06	0.111	6.02×10^{-01}
SCF	−0.23	0.049	3.01×10^{-06}	−0.31	0.098	1.78×10^{-03}
CDCP1	0.23	0.05	3.50×10^{-06}	0.42	0.101	4.06×10^{-05}
TNFSF14	0.22	0.049	4.80×10^{-06}	0.26	0.107	1.42×10^{-02}
TWEAK	−0.23	0.051	5.64×10^{-06}	−0.31	0.115	7.46×10^{-03}
IL-18R1	0.23	0.051	7.25×10^{-06}	0.26	0.116	2.58×10^{-02}
B—Cardiometabolic-related proteins						
CES1	0.66	0.05	4.91×10^{-40}	0.37	0.090	3.33×10^{-05}
F7	0.37	0.046	1.19×10^{-15}	0.08	0.106	4.36×10^{-01}
MET	−0.32	0.044	4.62×10^{-13}	−0.02	0.108	8.34×10^{-01}
SERPINA5	0.30	0.046	5.62×10^{-11}	−0.08	0.112	4.86×10^{-01}
KIT	−0.29	0.045	1.72×10^{-10}	−0.15	0.108	1.64×10^{-01}
THBS4	0.25	0.044	1.99×10^{-08}	0.33	0.103	1.39×10^{-03}
PLTP	−0.23	0.044	1.64×10^{-07}	−0.01	0.107	9.16×10^{-01}
PROC	0.22	0.044	6.19×10^{-07}	0.05	0.105	6.62×10^{-01}
FCN2	0.22	0.045	6.22×10^{-07}	0.41	0.106	1.23×10^{-04}
ICAM1	0.22	0.045	1.65×10^{-06}	0.24	0.112	3.38×10^{-02}
CNDP1	0.22	0.047	3.33×10^{-06}	−0.16	0.107	1.34×10^{-01}
MEGF9	0.20	0.043	4.52×10^{-06}	−0.22	0.099	2.64×10^{-02}
LILRB1	0.20	0.044	8.02×10^{-06}	0.27	0.101	7.78×10^{-03}
TGFBI	0.19	0.045	2.03×10^{-05}	0.42	0.110	1.31×10^{-04}
PTPRS	−0.18	0.043	2.28×10^{-05}	0.11	0.101	2.80×10^{-01}
CHL1	−0.18	0.043	3.03×10^{-05}	0.31	0.121	1.05×10^{-02}
SERPINA7	0.18	0.047	1.16×10^{-04}	−0.07	0.105	5.16×10^{-01}
TGFBR3	−0.16	0.042	1.30×10^{-04}	−0.06	0.106	5.89×10^{-01}
REG1A	−0.17	0.046	2.21×10^{-04}	−0.12	0.113	3.03×10^{-01}

Note: A and B are sorted based on Bonferroni-corrected *p*-value association of proteins with FLD and nominal *p*-value with liver fibrosis. Model 1 is adjusted only for age and sex and model 2 is further adjusted for RBCs, granulocyte, lymphocyte, monocyte, platelets, body mass index, smoking status, alcohol consumption, and type 2 diabetes. The Bonferroni-corrected significance threshold is $p<2.92\times10^{-4}$ (0.05/171 proteins) for hepatic steatosis, and the nominal association with $p<0.05$ for liver fibrosis.

Abbreviations: CDCP1, CUB domain-containing protein 1; CES1, carboxylesterase 1; CHL1, close homolog of L1; CNDP1, beta-Ala-His dipeptidase; CX3CL1, chemokine ligand 1; F7, Coagulation factor VII; FCN2, ficolin-2; FLD, fatty liver disease; ICAM1, intercellular adhesion molecules 1; IL-18R1, IL-18 receptor 1; KIT, proto-oncogene c-KIT; LILRB1, leukocyte immunoglobulin-like receptor subfamily B member 1; MEGF9, multiple EGF-like-domains 9; Met, tyrosine-protein kinase; PLTP, phospholipid transfer protein; PROC, protein C; PTPRS, Receptor-type tyrosine-protein phosphatase precursor; REG1A, regenerating protein type I subclass; SCF, stem cell factor; SERPINA5, serine protease inhibitor 5; SERPINA7, serine protease inhibitor 7; TGFBI, transforming growth factor, beta-induced, 68kDa; TGFBR3, transforming growth factor beta receptor III; THBS4, thrombospondin-4; TNFSF14, tumor necrosis factor superfamily member 14; TWEAK, TNF-related weak inducer of apoptosis.

gene expression frequency in 3-dimensional human liver-derived organoids. Of the investigated proteins, 25 out of 27 were tested in the liver organoids, and 16 could be detected in our model. Interestingly, the expression levels of *IL-18R1* and *CES1* were upregulated in the organoids mimicking FLD. The other inflammatory and cardiometabolic-related proteins could not be replicated in this FLD model (Table S7 http://links.lww.com/HEP/C647).

Plasma proteins and their potential as FLD biomarkers

Finally, we performed AUC analysis to test whether the identified proteins have the biomarker potential and could improve the prediction model of FLD. All prediction models demonstrated fair to good diagnostic performance for FLD: AUC: 0.763 (95% CI, 0.745–0.781) for proteomics alone, 0.788 (95% CI, 0.771–0.805) for FLI

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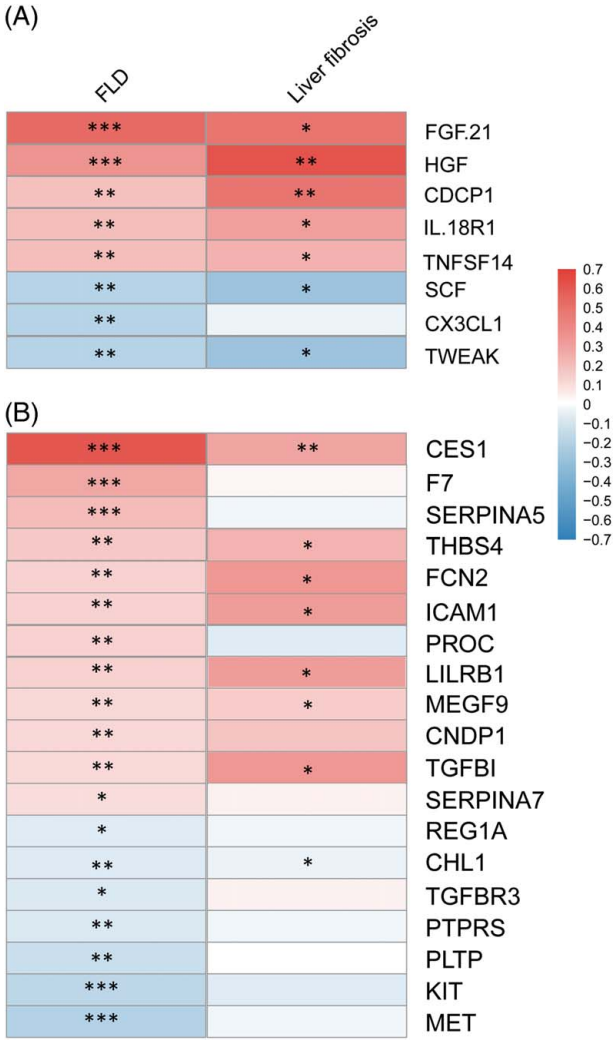

FIGURE 2 Heatmap visualization of protein profiles of FLD and liver fibrosis in inflammatory-related protein (A), cardiometabolic-related protein (B) based on the multilogistic regression model. Model 2 is adjusted for age, sex, blood cells count (RBCs, granulocyte, lymphocyte, monocyte, and platelets), body mass index, smoking status, alcohol consumption, and type2 diabetes. The color in columns represent the standardized effect estimates (betas) red color—positive relationship, blue color—negative relationship. *Significant relationship of proteins with FLD after adjusting for Bonferroni-correction ($p < 2.92\times10^{-4}$) and with liver fibrosis at the *p*-value of <0.05. *p*-value $<10^{-5}$(*), *p*-value between 10^{-5}–10^{-9} (**), and *p*-value $>10^{-9}$ (***). Abbreviations: CDCP1, CUB domain-containing protein 1; CES1, carboxylesterase 1; CHL1, close homolog of L1; CNDP1, beta-Ala-His dipeptidase; CX3CL1, chemokine ligand 1; F7, Coagulation factor VII; FCN2, ficolin-2; FLD, fatty liver disease; ICAM1, intercellular adhesion molecules 1; IL-18R1, IL-18 receptor 1; KIT, proto-oncogene c-KIT; LILRB1, leukocyte immunoglobulin-like receptor subfamily B member 1; MAFLD, metabolic associated fatty liver disease; MEGF9, multiple EGF-like-domains 9; Met, tyrosine-protein kinase; PLTP, phospholipid transfer protein; PROC, protein C; PTPRS, receptor-type tyrosine-protein phosphatase precursor; REG1A, regenerating protein type I subclass; SCF, stem cell factor; SERPINA5, serine protease inhibitor 5; SERPINA7, serine protease inhibitor 7; TGFBI, transforming growth factor, beta-induced, 68kDa; TGFBR3, transforming growth factor beta receptor III; THBS4, thrombospondin-4; TNFSF14, tumor necrosis factor superfamily member 14; TWEAK, TNF-related weak inducer of apoptosis.

alone, and 0.806 (95% CI, 0.790–0.822) for FLI combined with proteomics. Although proteomics alone had inferior diagnostic accuracy compared with the FLI, adding proteomics to the FLI algorithm slightly improved the AUC from 0.788 to 0.806 (Figure 3A), but this was not statistically significant. Regarding fibrosis, proteomics alone (AUC: 0.758, 95% CI, 0.720–0.796) significantly outperformed the FIB-4 (AUC: 0.634, 95% CI, 0.584–0.684), and combining proteomics with FIB-4 yielded the best results (AUC: 0.779, 95% CI, 0.743–0.815 (Figure 3B)], but not significantly better than the proteomics alone.

DISCUSSION

We investigated the association between 171 plasma proteins and FLD among a large population-based cohort study. We found 8 inflammatory and 19 cardiometabolic-related proteins to be significantly associated with FLD, of which FGF-21, CES1, and IL-18R1 will be discussed to a greater extent here, whereas the remaining proteins are briefly discussed in Table S8 (http://links.lww.com/HEP/C647). Several of the proteins associated with FLD were also associated with fibrosis. These findings are important for the further unraveling of the pathophysiology of FLD and the progression toward fibrosis. Moreover, these findings imply that plasma protein levels may have the potential to be used as biomarkers.

Higher plasma level of FGF-21 was associated with an increased risk of FLD and liver fibrosis and, therefore, may play a crucial role in the pathophysiology of the disease and its progression toward fibrosis. FGF-21 is known for its role in many metabolic processes, including insulin sensitivity, glucose and lipid metabolism, and energy homeostasis.[21] Key aspects are increased with GLUT1 expression through stimulation of FGF-21, which induces glucose absorption in adipocytes.[22] Despite we could not validate this finding in liver organoids (due to not being able to reach the detection limit in FLD mimicking organoids and controls), it is supported by various previous studies that explored the association of plasma inflammatory and cardiometabolic proteins with FLD. For example, the expression of FGF-21 was an independent predictor of NAFLD, and plasma levels of FGF-21 reflect fat accumulation and dysregulation of metabolic pathways in the liver.[23,24] Interestingly, FGF-21 has been shown previously to be associated with FLD progression. For example, FGF-21 was related to lobular inflammation in patients with NAFLD, independent of steatosis grade and BMI.[25] Moreover, serum FGF-21 expression increased with the severity of liver fibrosis.[26] Overall, there is emerging evidence showing that FGF-21 is an important link to FLD and fibrosis. Our study highlights the relevance of these concepts in the general population.

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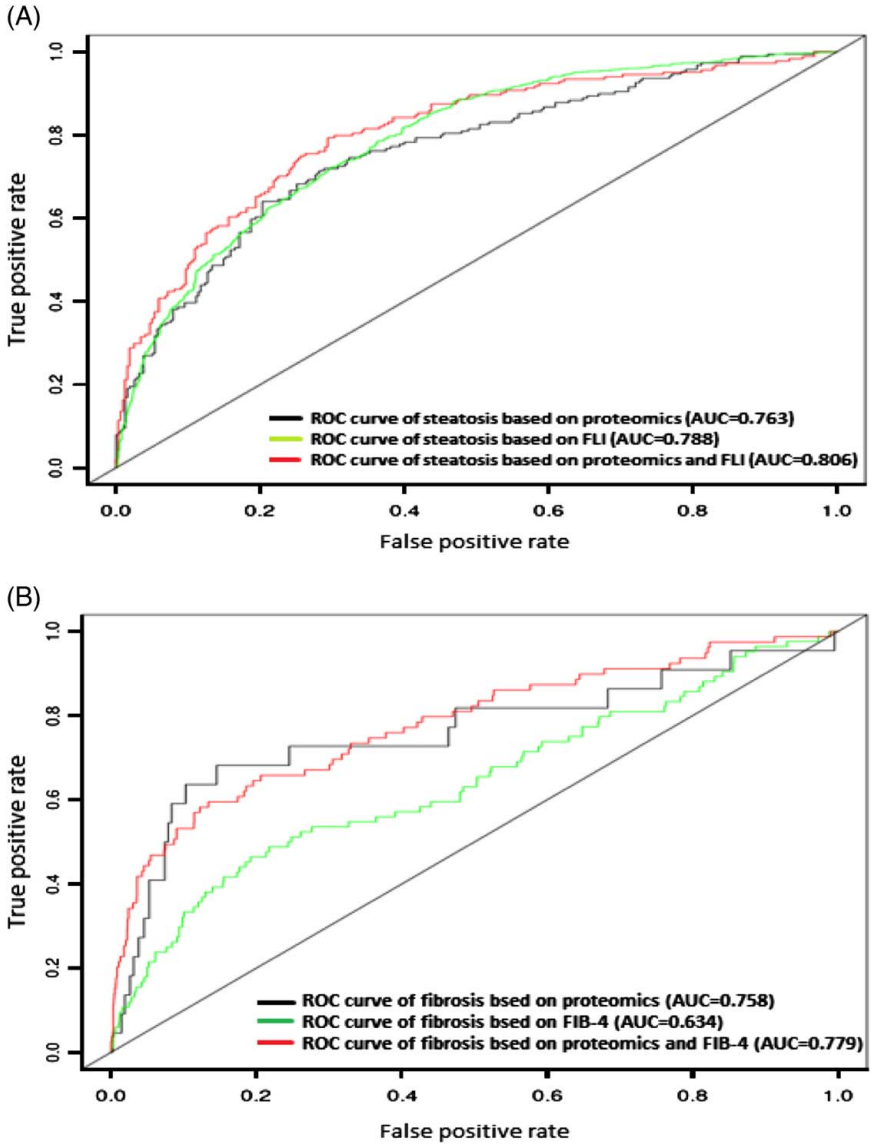
(A)
True positive rate
False positive rate
ROC curve of steatosis based on proteomics (AUC=0.763)
ROC curve of steatosis based on FLI (AUC=0.788)
ROC curve of steatosis based on proteomics and FLI (AUC=0.806)
(B)
True positive rate
False positive rate
ROC curve of fibrosis bsed on proteomics (AUC=0.758)
ROC curve of fibrosis bsed on FIB-4 (AUC=0.634)
ROC curve of fibrosis bsed on proteomics and FIB-4 (AUC=0.779)

FIGURE 3 AUC-ROC curve of each prediction model for predicting fatty liver disease (A) and fibrosis (B). Abbreviations: FLI, fatty liver index; ROC, receiver operator characteristics.

Another important protein is CES1, for which upregulation was significantly associated with a higher risk of FLD and fibrosis. In liver organoids, we also demonstrated the upregulation of CES1 in the models mimicking FLD, aligning our main outcomes. CES1 is involved in catalyzing the hydrolysis of esters, amides, thioesters, and carbamates, through which CES1 is important in drug metabolism.[27] Despite few studies being available that investigated the correlation between CES1 protein and FLD, the expression of *CES1* gene has been linked to FLD in humans.[28] This finding aligns with the results obtained in mouse models that demonstrated CES1 might be a potential treatment target for hyperlipidemia and NAFLD management.[29] Further research is warranted to unravel the role of CES1 in the pathogenesis of NAFLD and fibrosis and whether it could improve the current noninvasive assessment of fibrosis.

Moreover, we found that the upregulation of IL-18R1 is associated with FLD and fibrosis, which was further validated in our experimental study with liver organoids. IL-18R1 specifically binds IL-18 and is essential for IL-18-mediated signal transduction.[30] IL-18 is an important inflammatory cytokine, which initiates a signaling complex by binding to the IL-18 alpha chain (IL-18Rα) and a coreceptor, termed IL-18 receptor beta chain (IL-18Rβ), forming a high-affinity complex.[31] Many studies have primarily focused on the role of ligand (IL-18) in FLD rather than the receptor; however, a previous study in humans found that IL-18R1 was significantly correlated with a specific pattern of fat depots in the liver.[32] Moreover, in an animal model, IL-18R-dependent signaling was identified as a modulator of early liver

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damage among subjects with FLD. Interestingly, this could already be detected preceding the development of histologic NASH through activation of the NLR family pyrin domain-containing-3 (NLRP3).[33] Together with IL-12, IL-18 may cause impairment of the hepatic microcirculation, creating an ischemic state in the liver and inhibiting oxidative phosphorylation.[34] It has also been shown that IL-18 could predict advanced FLD in children.[35] Validation of these results is warranted, and further investigation is required on the interactions between IL-18 and its receptor (IL-18R1) in FLD and their potential as disease biomarkers.

To link our findings to protein cascades, we also performed pathway analysis. These results indicated that TWEAK and TNFSF14 belong to the noncanonical NF-κB signaling pathway, which regulates inflammation and host immune response.[36] In an animal model, both FGF-inducible 14 (Fn14) and TWEAK in knockout mice and wild-type mice showed proliferation in hepatocytes and cholangiocytes after partial hepatectomy, indicating that the proteins signal is essential for mouse liver regeneration after hepatectomy.[36,37] It has also been shown that TNFSF14, which increases adipose tissue inflammation, responds through its interaction with HVEM (herpes simplex virus glycoprotein D for herpes virus entry mediator).[38]

Moreover, we found F7, SERPINA5, and PROC to be involved in the fibrin clot formation pathways. Among them, SERPINA5 protein is involved in the common fibrin clot formation pathways through inactive complexes with protein C inhibitor that inhibits thrombin in complex with thrombomodulin.[39] F7 is a vitamin K–dependent glycoprotein formed by the liver, which can initiate the extrinsic pathway of blood coagulation.[40] The vitamin K–dependent protein C (PROC) pathway exerts its anticoagulant circulation properties as a proenzyme to an anticoagulant serine protease and is activated by thrombin.[41] Although clearly related to the liver, these identified pathways warrant further explanation as it remains unclear how exactly these protein cascades are linked to FLD and disease progression.

Finally, our AUC-analyses illustrated that proteomics has the potential to improve currently available noninvasive markers (FLI and FIB-4) in the prediction of FLD or fibrosis. Although we identified a slight improvement in the prediction models, the illustrated potential of proteomics in noninvasive tests aligns with previous studies in small cohorts.[9,10]

This study has several important strengths, including the large sample size, use of proximity extension assay technology, and availability of liver stiffness data in addition to ultrasound, yet the following limitations need to be considered for the interpretation of our findings. First, collecting data on FLD was 5.54 (p25–p75: 5.4–5.7) years after the plasma sample collection for the proteomic assessment. However, the impact on our results might be limited, as the time gap would result in "dilution toward the null" and would make it increasingly difficult to demonstrate associations. Nonetheless, this may have resulted in missing out on some proteins associated with FLD and fibrosis. Reassuringly, an additional analysis using an FLI-based definition of FLD could confirm 25 out of 27 proteins that were identified in the main analysis. Second, we used surrogate markers for FLD and fibrosis, since it is unethical to perform the liver biopsy in apparently healthy volunteers exposing them to risk of serious complications. Third, our results warrant validation in future studies, as only few studies with different study designs are available to relate to our current findings. Aiming at the validation, we used liver organoids; however, not all identified proteins could be validated. This may partially be attributed to differences in protein assessment but also due to the fact that liver organoids cannot entirely mimic the total protein expression in humans as was measured in our cohort study. Despite the differences, it was reassuring that CES1 and IL-18R1 were validated, providing strong evidence that these proteins play an important role in FLD and its progression to fibrosis. Fourth, because of the nature of this study, we could not address causality. Lastly, this study did not contain longitudinal analysis because multiple measurements of the proteomics and outcome were not available in the follow-up visits.

In conclusion, we demonstrate that plasma levels of several inflammatory and cardiometabolic proteins are significantly associated with FLD and fibrosis at a population level. Our findings may help better understand FLD's pathophysiology and the disease progression into fibrosis. In particular, FGF-21, CES1, and IL-18R1 seem to contribute to the pathogenesis of FLD and also fibrosis. Moreover, given an unmet need for accurate and widely available noninvasive tools for risk stratification and referral strategies, our findings may improve the algorithms currently under development.

AUTHOR CONTRIBUTIONS

Mohsen Ghanbari: supervised and designed the research. Yasir J. Abozaid: analyzed the data and wrote the manuscript. Robert J. de Knegt, Willem P. Brouwer and Laurens A. van Kleef: provided the fatty liver data and consultation regarding the results of liver data. Ibrahim Ayada and Quiwe Pan conducted the in-vitro expriments in liver organoids. Ibrahim Ayada and Laurens A. van Kleef: discussed the results and contributed to the manuscript writing. Costanza L. Vallerga and Joyce Van Meurs: provided consultation regarding the proteomics data in the Rotterdam Study. Mohsen Ghanbari, Ibrahim Ayada, Laurens A. van Kleef, Costanza L Vallerga, Joyce Van Meurs, M. Arfan Ikram, Qiuwei Pan, Willem P. Brouwer, and Robert J. de Knegt: critical review of the manuscript and approval of the final version.

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ACKNOWLEDGMENTS

The authors thank the contribution of participants, staff, general practitioners, and pharmacists in the Rotterdam Study. The authors also thank Ingrid Szilagyi for the help with the QC of the generated proteomic dataset.

FUNDING INFORMATION

The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, the Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. The proteomics data generation was funded by the Netherlands Organisation for Health Research and Development (ZonMw), project number 849200003. This project was partly supported by the Erasmus MC Fellowship (EMCF20213) grant of Mohsen Ghanbari. The mentioned funders had no role in the design and conduct of the study, nor in the decision to submit the manuscript for publication.

CONFLICTS OF INTEREST

Robert J. de Knegt consults for, advises, is on the speakers' bureau for, and received grants from Abbvie. He advises and received grants from Gilead. He is on the speakers' bureau and received grants from Echosens. He received grants from GSK. The remaining authors have no conflicts to report.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this articles. The RS data can be made available to interested researchers upon request. Requests can be directed to data manager Frank J. A. van Rooij (f.vanrooij@erasmusmc.nl) or visit the following website for more information: https://www.ergo-onderzoek.nl/contact. We are unable to place data in a public repository due to legal and ethical constraints. Sharing of individual participant data was not included in the informed consent of the study and there is potential risk of revealing participants' identities, as it is not possible to completely anonymize the data.

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REFERENCES

1. Le MH, Yeo YH, Li X, Li J, Zou B, Wu Y, et al. 2019 global NAFLD prevalence: a systematic review and meta-analysis. Clin Gastroenterol Hepatol . 2021. Epub ahead of print.
2. Wong RJ, Ahmed A. Obesity and non-alcoholic fatty liver disease: disparate associations among Asian populations. World J Hepatol. 2014;6:263.
3. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67:328–57.
4. Monetti M, Levin MC, Watt MJ, Sajan MP, Marmor S, Hubbard BK, et al. Dissociation of hepatic steatosis and insulin resistance in mice overexpressing DGAT in the liver. Cell Metab. 2007;6:69–78.
5. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med . 2002;346:1221–31.
6. Younossi ZM, Blissett D, Blissett R, Henry L, Stepanova M, Younossi Y, et al. The economic and clinical burden of nonalcoholic fatty liver disease in the United States and Europe. Hepatology. 2016;64:1577–86.
7. Lazarus JV, Mark HE, Anstee QM, Arab JP, Batterham RL, Castera L, et al. Advancing the global public health agenda for NAFLD: a consensus statement. Nat Rev Gastroenterol Hepatol. 2022;19:60–78.
8. Niu L, Thiele M, Geyer PE, Rasmussen DN, Webel HE, Santos A, et al. Noninvasive proteomic biomarkers for alcohol-related liver disease. Nat Med. 2022;28:1–11.
9. Corey KE, Pitts R, Lai M, Loureiro J, Masia R, Osganian SA, et al. ADAMTSL2 protein and a soluble biomarker signature identify at-risk non-alcoholic steatohepatitis and fibrosis in adults with NAFLD. J Hepatol. 2022;76:25–33.
10. Luo Y, Wadhawan S, Greenfield A, Decato BE, Oseini AM, Collen R, et al. SOMAscan proteomics identifies serum biomarkers associated with liver fibrosis in patients with NASH. Hepatol Commun . 2021;5:760–3.
11. Ikram MA, Brusselle G, Ghanbari M, Goedegebure A, Ikram MK, Kavousi M, et al. Objectives, design and main findings until 2020 from the Rotterdam Study. Eur J Epidemiol. 2020;35:483–517.
12. Hamaguchi M, Kojima T, Itoh Y, Harano Y, Fujii K, Nakajima T, et al. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. Am J Gastroenterol. 2007;102:2708–15.
13. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. J Hepatol. 2020;73:202–9.
14. Boursier J, Vergniol J, Guillet A, Hiriart J-B, Lannes A, Le Bail B, et al. Diagnostic accuracy and prognostic significance of blood fibrosis tests and liver stiffness measurement by FibroScan in non-alcoholic fatty liver disease. J Hepatol. 2016;65:570–8.
15. Roulot D, Costes JL, Buyck JF, Warzocha U, Gambier N, Czernichow S, et al. Transient elastography as a screening tool for liver fibrosis and cirrhosis in a community-based population aged over 45 years. Gut. 2011;60:977–84.

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16. PEA – a high-multiplex immunoassay technology with qPCR or NGS readout, 2020.
17. Szilagyi IA, Vallerga CL, Boer CG, Schiphof D, Ikram MA, Bierma-Zeinstra SMA, et al. Plasma proteomics identifies CRTAC1 as a biomarker for osteoarthritis severity and progression. Rheumatology (Oxford). 2022;62:1286–95.
18. World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. 2006.
19. Wang L, Li M, Yu B, Shi S, Liu J, Zhang R, et al. Recapitulating lipid accumulation and related metabolic dysregulation in human liver-derived organoids. J Mol Med. 2022;100:471–84.
20. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol. 2006;6:1–7.
21. Tucker B, Li H, Long X, Rye K-A, Ong KL. Fibroblast growth factor 21 in non-alcoholic fatty liver disease. Metabolism. 2019; 101:153994.
22. Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, et al. FGF-21 as a novel metabolic regulator. J Clin Invest. 2005;115:1627–35.
23. Rusli F, Deelen J, Andriyani E, Boekschoten MV, Lute C, van den Akker EB, et al. Fibroblast growth factor 21 reflects liver fat accumulation and dysregulation of signalling pathways in the liver of C57BL/6J mice. Sci Rep. 2016;6:1–16.
24. Li H, Dong K, Fang Q, Hou X, Zhou M, Bao Y, et al. High serum level of fibroblast growth factor 21 is an independent predictor of non-alcoholic fatty liver disease: a 3-year prospective study in China. J Hepatol. 2013;58:557–63.
25. Shen J, Chan HL-Y, Wong GL-H, Choi PC-L, Chan AW-H, Chan H-Y, et al. Non-invasive diagnosis of non-alcoholic steatohepatitis by combined serum biomarkers. J Hepatol. 2012;56:1363–70.
26. Li D, Lu T, Shen C, Liu Y, Zhang J, Shan Y, et al. Expression of fibroblast growth factor 21 in patients with biliary atresia. Cytokine. 2016;83:13–8.
27. Casey Laizure S, Herring V, Hu Z, Witbrodt K, Parker RB. The role of human carboxylesterases in drug metabolism: have we overlooked their importance? Pharmacotherapy. 2013;33:210–2.
28. Ashla AA, Hoshikawa Y, Tsuchiya H, Hashiguchi K, Enjoji M, Nakamuta M, et al. Genetic analysis of expression profile involved in retinoid metabolism in non-alcoholic fatty liver disease. Hepatol Res. 2010;40:594–604.
29. Lian J, Bahitham W, Panigrahi R, Nelson R, Li L, Watts R, et al. Genetic variation in human carboxylesterase CES1 confers resistance to hepatic steatosis. Biochim Biophys Acta Mol Cell Biol Lipids. 2018;1863:688–99.
30. Guo F, Wu S. Antisense IRAK-1 oligonucleotide blocks activation of NF-κB and AP-1 induced by IL-18. Immunopharmacology. 2000;49:241–6.
31. Dinarello CA, Novick D, Kim S, Kaplanski G. Interleukin-18 and IL-18 binding protein. Front Immunol. 2013;4:289.
32. Lovric A, Granér M, Bjornson E, Arif M, Benfeitas R, Nyman K, et al. Characterization of different fat depots in NAFLD using inflammation-associated proteome, lipidome and metabolome. Sci Rep. 2018;8:1–14.
33. Hohenester S, Kanitz V, Schiergens T, Einer C, Nagel J, Wimmer R, et al. IL-18 but not IL-1 signaling is pivotal for the initiation of liver injury in murine non-alcoholic fatty liver disease. Int J Mol Sci. 2020;21:8602.
34. Kaneda M, Kashiwamura S-I, Ueda H, Sawada K, Sugihara A, Terada N, et al. Inflammatory liver steatosis caused by IL-12 and IL-18. J Interferon Cytokine Res. 2003;23:155–62.
35. Flisiak-Jackiewicz M, Bobrus-Chociej A, Tarasów E, Wojtkowska M, Białokoz-Kalinowska I, Lebensztejn DM. Predictive role of interleukin-18 in liver steatosis in obese children. Can J Gastroenterol Hepatol . 2018;2018::3870454.
36. Chen Q, Lu X, Zhang X. Noncanonical NF-κB signaling pathway in liver diseases. J Clin Transl Hepatol. 2021;9:81.
37. Karaca G, Swiderska-Syn M, Xie G, Syn W-K, Krüger L, Machado MV, et al. TWEAK/Fn14 signaling is required for liver regeneration after partial hepatectomy in mice. PLoS One. 2014; 9:e83987.
38. Kim H-M, Jeong C-S, Choi H-S, Kawada T, Yu R. LIGHT/TNFSF14 enhances adipose tissue inflammatory responses through its interaction with HVEM. FEBS Lett. 2011;585:579–84.
39. Davie EW, Fujikawa K, Kisiel W. The coagulation cascade: initiation, maintenance, and regulation. Biochemistry. 1991;30:10363–70.
40. Manzano ML, Arocena C, Tomas JF, Hedner U, Quiroga JA, Carreno V. Measurement of the procoagulant activity of factor VII in patients with liver cirrhosis and normal prothrombin activity: evaluation of the bleeding risk. Blood Coagul Fibrinolysis. 2000;11:S95–99.
41. Dahlbäck B, Villoutreix BO. The anticoagulant protein C pathway. FEBS Lett. 2005;579:3310–6.

How to cite this article: Abozaid YJ, Ayada I, van Kleef LA, Vallerga CL, Pan Q, Brouwer WP, et al. Plasma proteomic signature of fatty liver disease: The Rotterdam Study. Hepatology. 2023;78:284–294. https://doi.org/10.1097/HEP.0000000000000300

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